A fast gas chromatographic method for the study of semiochemical slow release formulations

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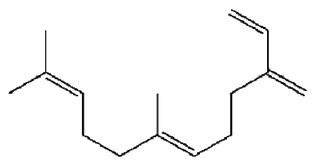
Introduction

E-*β*-Farnesene, the alarm pheromone of many aphid species and *β*-caryophyllene, recently identified as one of the possible component of the aggregation pheromones of the Asian ladybeetles *Harmonia axyridis* Pallas, are considered as two sesquiterpenes attractive for aphids' predators and parasitoids.

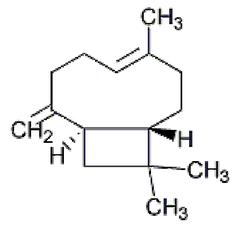
In the present research, a fast GC method was optimised for the quantitative characterisation, in less than five minutes, of the above mentioned semiochemicals formulated in slow release devices. The formulations were evaluated in terms of volatiles release capacity and protection efficiency of sesquiterpenes. The sesquiterpenes used in the formulations were obtained from natural sources. They were purified by flash chromatography fractionation (under pressure) of essential oils.

The fast chromatographic method was completely validated by means of the accuracy profile concept.

E-β-farnesene



β-caryophyllene



Experimental

Flash Chromatography fractionation of essential oils

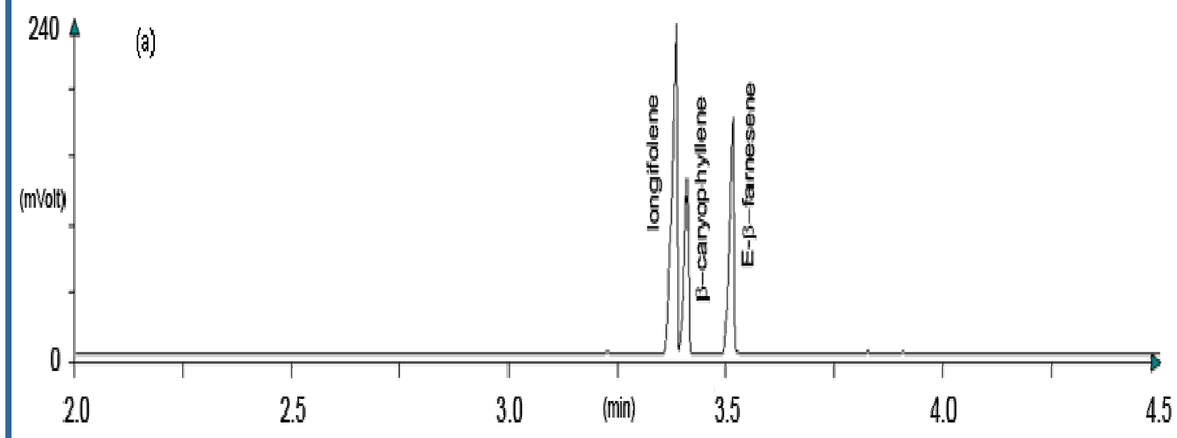
Matricaria chamomilla

Compounds	
Sum of monoterpenes	1.3 %
E-β-farnesene	**83.8 %**
Germacrene D	1.5 %
Bicyclogermacrene	1.5 %
(E,E)-α-farnesene	11.9 %

Nepeta cataria

Compounds	
Sum of monoterpenes	1.2 %
β-caryophyllene	**97.7 %**
α-humulene	1.1 %

Fast GC analytical method



Calculated **selectivity factor (*α*)** between longifolene and *β*-caryophyllene : **1.016 > 1**

Chromatographic conditions

Ultra Fast Module : Ph5; 0.1 µm film thickness, 5m x 0.1mm I.D.

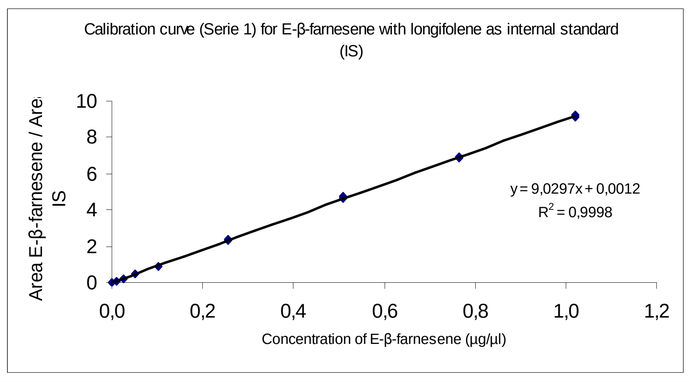
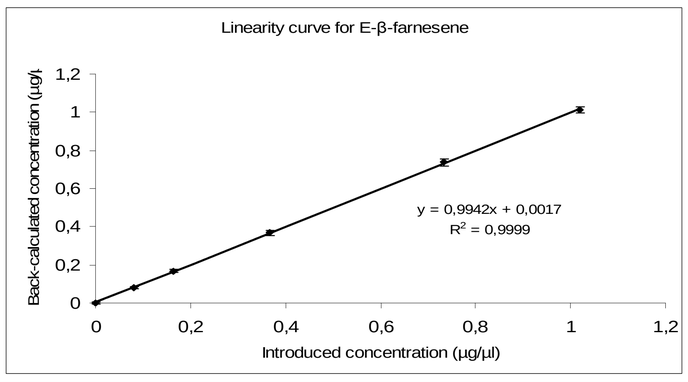
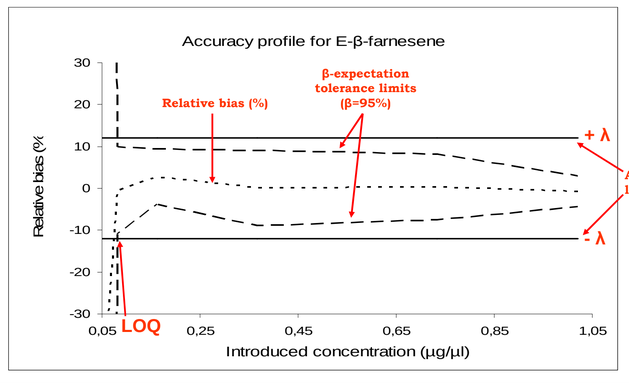
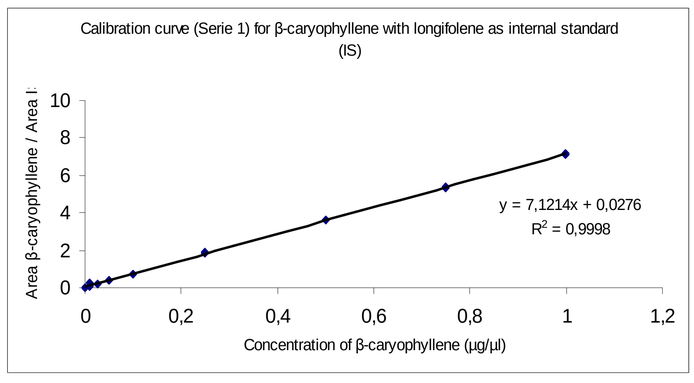
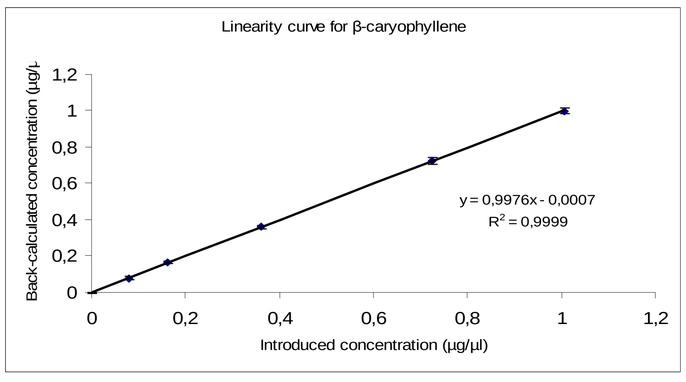
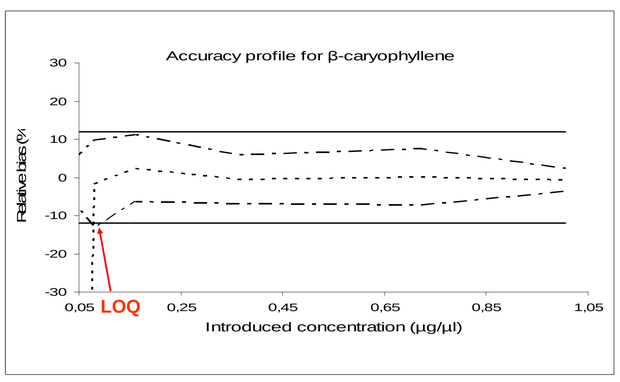
Carrier gas : He; 0.5 ml/min

Split ratio : 1:100

Oven :

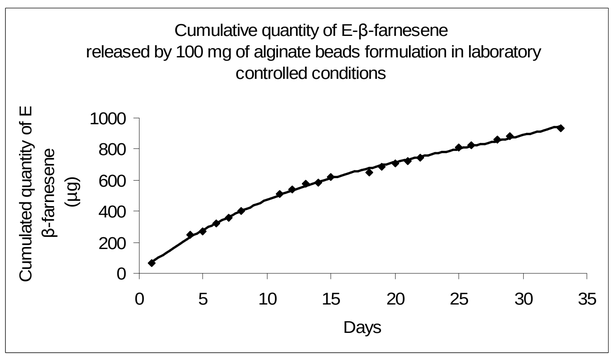
Initial T° : 40°C; 0.10 min
Ramp 1 : 30°C/min ➔ 95°C
Ramp 2 : 35°C/min ➔ 155°C
Ramp 3 : 200°C/min ➔ 280°C; 0.5 min
Oven run time : **4.78 min**

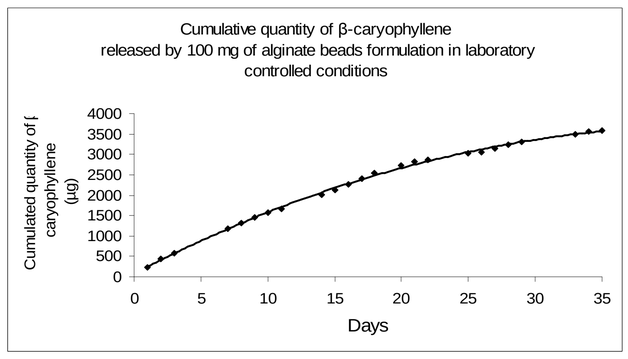
Validation by accuracy profile

	Calibration standards	***Validation standards***	***Accuracy Profile***
	Linear regression models	Back-calculation of the results ➔ linearity curves	**ACCURACY = TRUENESS + PRECISION**
E-β-farnesene			
β-caryophyllene			

Applications

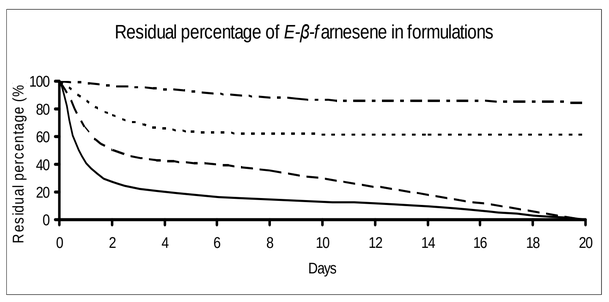
Volatile release capacity of formulations

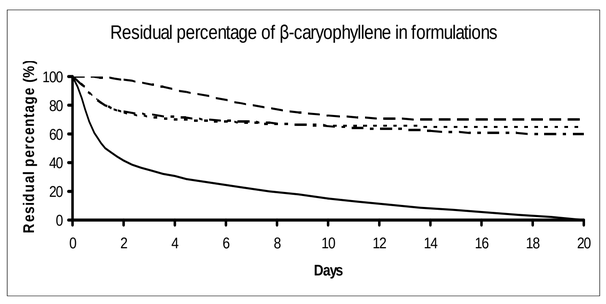




Laboratory controlled conditions :
- Temperature : 20°C
- Sampling air flow : 0.5 L/min
- Relative humidity : 65 %

Sesquiterpenes protection efficiency of formulations





Alginate gel beads without alpha-tocopherol
Alginate gel beads with alpha-tocopherol
Sunflower oil formulation
Purified compounds non formulated

Conclusions

- Fast achievement of high purity semiochemicals by flash chromatography of essential oils.
- Fast GC method for characterization and quantification of sesquiterpenes in less than 5 minutes for various applications. Moreover the good analytical performances of the fast column were demonstrated (see *Heuskin et al.,* 2009, J. of Chromatography A, 1216, pp 2768-2775).
- Method completely validated for the two sesquiterpenes by the accuracy profile concept considering a risk of 5 % and a linear regression model.
- Low limits of quantification